Acta Chromatographica 20(2008)3, 411–421
DOI: 10.1556/AChrom.20.2008.3.8

Simultaneous Analysis of Ambroxol HCl with Cetirizine HCl and of Ambroxol HCl with *levo*-Cetirizine Dihydrochloride in Solid Dosage Forms by RP-HPLC

A.S. BIRAJDAR*, S.N. MEYYANATHAN, R.B. RAJA, N. KRISHANAVENI, AND B. SURESH

Department of Pharmaceutical Analysis, J.S.S. College of Pharmacy,
Post Box 20, Rocklands, Ooty-643001, Tamilnadu, India
E-mail: aruna_birajdar@rediffmail.com

Summary. An isocratic RP-HPLC method has been developed and validated for simultaneous analysis of ambroxol hydrochloride (AMB) with cetirizine hydrochloride (CTZ) and of ambroxol hydrochloride (AMB) with *levo*-cetirizine dihydrochloride (LCTZ) in combined solid dosage forms. Formulations containing AMB with CTZ (tablets) and AMB with LCTZ (capsules) are used as antihistaminic H_1 blockers. Chromatography was performed on a 250 mm × 4.6 mm, 5-µm particle size, C_{18} (ODS) column with a 45:30:30 (*v*/*v*) mixture of 30 mM aqueous ammonium sulphate (pH 5.5), acetonitrile, and methanol as mobile phase at a flow rate of 1 mL min^{-1}. The detection wavelength was 230 nm and analysis was performed at room temperature. Hydrochlorothiazide was used as internal standard for both formulations. Plots of drug-to-internal standard peak-area ratios (response factor) against respective concentrations were linear in the range 3 to 20 µg mL^{-1} for AMB and in the range 1 to 11 µg mL^{-1} for CTZ and LCTZ. The method was precise (RSD < 2) and accurate for analysis of both drugs in pharmaceutical dosage forms. Statistical data and results from recovery studies were reported for both formulations.

Key Words: ambroxol HCl, cetirizine HCl, *levo*-cetirizine dihydrochloride, hydrochlorothiazide, RP-HPLC

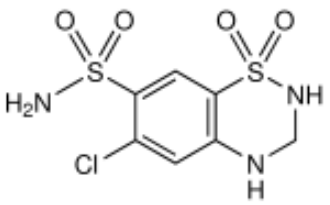
Introduction

Ambroxol hydrochloride (AMB) is an expectorant and mucolytic agent. Chemically it is *trans*-4-(2-amino-3,5-dibromobenzyl)aminocyclohexanol hydrochloride. Cetirizine hydrochloride (CTZ) is a histamine H1 receptor agonist. It is piperazine derivative and metabolite of hydroxyzine. Chemically it is 2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy] ace-

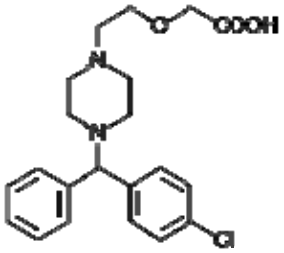
tic acid dihydrochloride [1]. *levo*-Cetirizine, the *R* enantiomer of cetirizine, is a second-generation H1 antagonist selective and potent for treatment of allergic rhinitis and chronic idiopathic urticaria [2]. Combinations of AMB with CTZ and AMB with LCTZ in drug formulations used as antihistaminic H1 blockers.

A literature survey reveals that several methods, including derivative UV spectrophotometry and HPLC [3], has been reported for individual estimation of AMB in tablets. Analysis of AMB in formulations has been performed by HPLC [4] and CTZ has been analysed by UV-derivative spectrophotometry [5]. CTZ have been analysed by UV and HPLC [6]. CTZ in formulations has been analysed by HPLC [7–10]. CTZ in human plasma has been analysed by HPLC [11–13]. LC–MS has been reported for determination of LCTZ [14]. No method has been reported for simultaneous estimation of AMB with CTZ or AMB with LCTZ in combined solid dosage forms, however. In this paper a simple and reproducible RP-HPLC method for simultaneous estimation of AMB with CTZ and of AMB with LCTZ, in combined forms, is reported. AMB is normally formulated in combination with CTZ or the more potent *R* isomer, LCTZ. The method was validated by following the ICH guidelines [15–17].

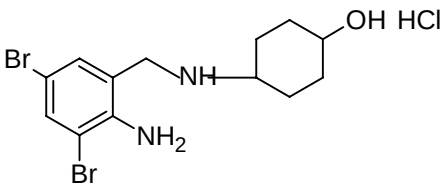
The chemical structures of ambroxol HCl, cetirizine HCl, *levo*-cetirizine, and hydrochlorothiazide, used as internal standard (IS) in this work, are shown in *Fig. 1*.



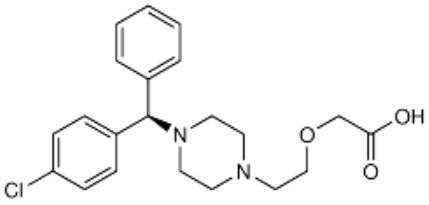
Hydrochlorothiazide (IS)



Cetirizine



Ambroxol hydrochloride



Levo-cetirizine

Fig. 1. The chemical structures of ambroxol HCl, cetirizine HCl, *levo*-cetirizine, and hydrochlorothiazide

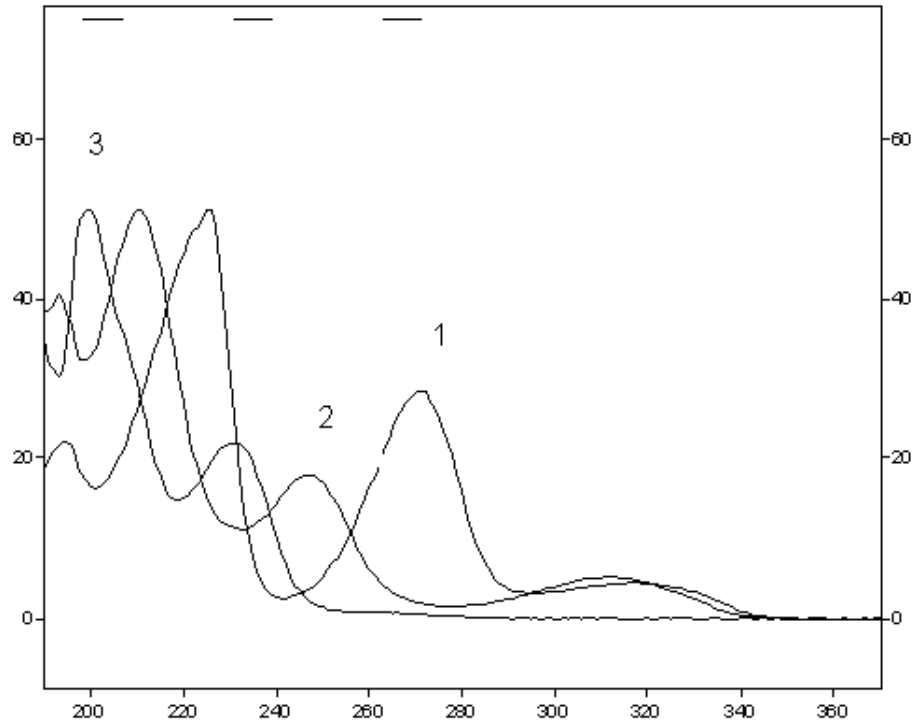


Fig. 2. Overlain UV spectra of: (1) hydrochlorothiazide (IS), (2) ambroxol HCl, and (3) cetirizine HCl/*levo*-cetirizine diHCl

Experimental

Chemicals and Reagents

HPLC-grade acetonitrile and methanol were procured from E. Merck (Mumbai, India) and pure standards of ambroxol HCl (99.68%) and cetirizine HCl (99.80%) were procured from Franco-Indian Pharma (Mumbai, India). *levo*-Cetirizine dihydrochloride (99.55%) and hydrochlorothiazide (99.42%; IS) were procured from Apex Pharmaceuticals. Ammonium sulphate, analytical grade, was procured from Qualigens Fine Chemicals (Mumbai, India). HPLC-grade water was from a Milli-QRO water-purification system.

Instrumentation and Chromatographic Conditions

Chromatography was performed with a Shimadzu (Japan) liquid chromatograph comprising an LC-10AT-vp solvent-delivery system (pump), an SPD M-10AVP photodiode-array detector, and a Rheodyne 7725i injector with 20-µL loop. A Class-VP 6.01 data station (Shimadzu) was used for data collection and processing. A Phenomenex C18 column (250 cm × 4.6 mm i.d., 5-µm particle) was used for chromatographic separation under suitable conditions.

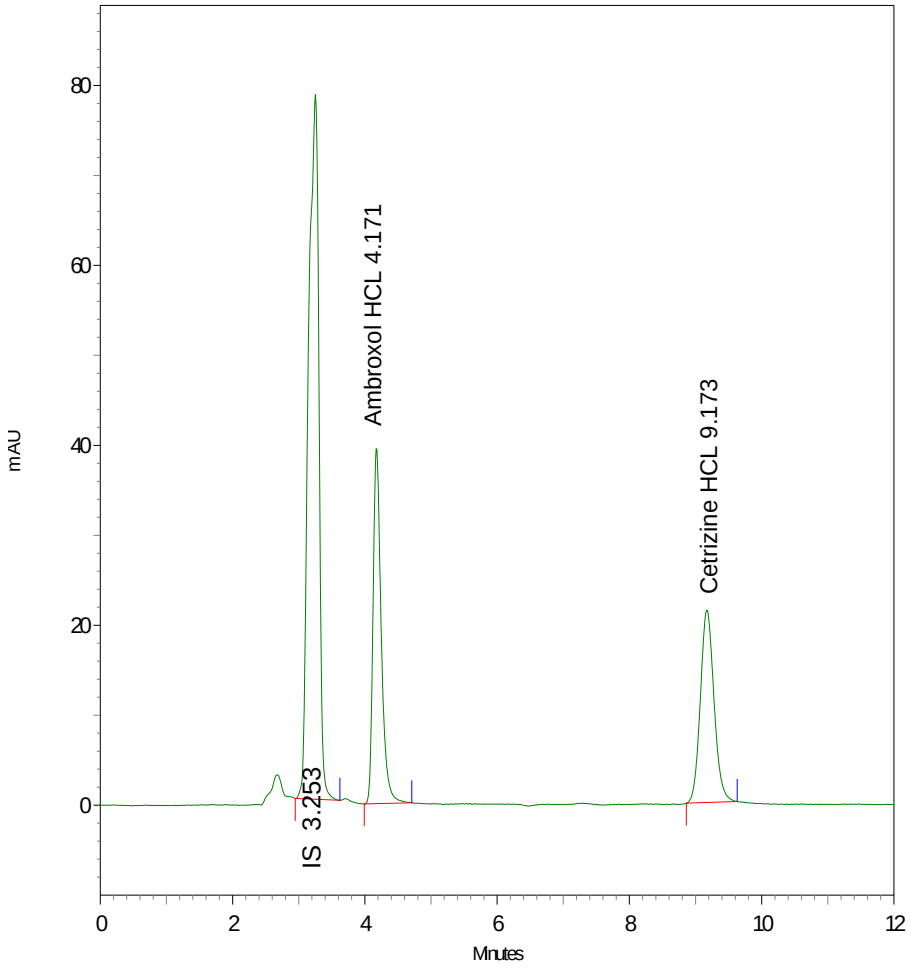


Fig. 3. Typical chromatogram obtained from hydrochlorothiazide (IS), ambroxol HCl, and cetirizine HCl/*levo*-cetirizine diHCl

The mobile phase was a 45:30:30 (*v*/*v*) mixture of 30 mM aqueous ammonium sulphate (pH 5.5), acetonitrile, and methanol at a flow rate of 1 mL min^{-1}. The detection wavelength was 230 nm at which both drugs and the IS absorb, as shown in *Fig. 2*. The peaks were identified by retention time, in comparison with those of standards, and by use of characteristic spectra acquired using the photodiode-array detector. A typical chromatogram is shown in *Fig. 3*.

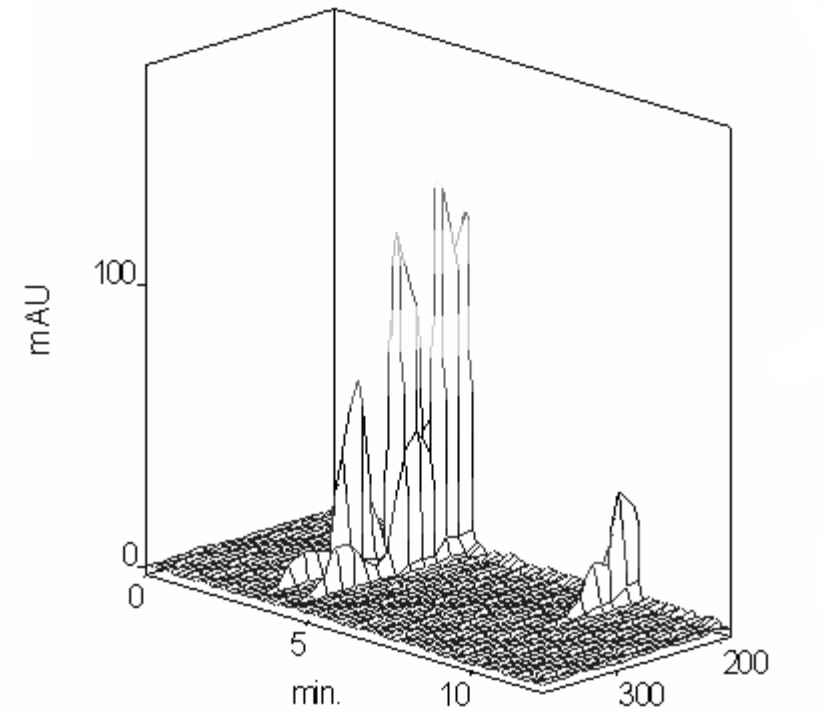


Fig. 4. 3D chromatogram obtained from hydrochlorothiazide (IS), ambroxol HCl, and cetirizine HCl/*levo*-cetirizine diHCl

Standard Solutions for Calibration Plots

Standard stock solutions (1 mg mL^{-1}) of AMB, CTZ, and LCTZ were prepared separately in a 1:1 (*v*/*v*) mixture of methanol and water. The stock solution of AMB was diluted with the mobile phase to give working standard solutions containing 3–20 µg mL^{-1}. Similarly the CTZ and LCTZ stock solutions were diluted with the mobile phase to give working standard solutions in the range 1–11 µg mL^{-1}. Hydrochlorothiazide, as internal standard (IS), was added at a constant level of 10 µg mL^{-1} to all the working standard solutions. These standard solutions were injected for construction of calibra-

tion plots by plotting drug-to-IS peak-area ratio (y) for each of the drugs against concentration (x). Analysis was performed at ambient temperature. The retention times of hydrochlorothiazide, AMB, CTZ, and LCTZ under these conditions were 3.25, 4.17, 9.17, and 9.17 min respectively.

Assay Procedure

Twenty Relent tablets, each containing AMB (60 mg) and CTZ (5 mg) were weighed, finely powdered, and an amount of powder equivalent to one tablet was extracted with a 1:1 (v/v) mixture of water and methanol (2 × 20 mL) and the extracts were combined and diluted to 100 mL. Twenty Laveta-A capsules, each containing AMB (75 mg) and LCTZ (5 mg) were treated in the same way.

The excipients were separated by filtration. Further dilution with mobile phase furnished concentrations of 12 µg mL^{-1} for AMB and 1 µg mL^{-1} for CTZ from tablets, and 15 µg mL^{-1} for AMB and 1 µg mL^{-1} for LCTZ from capsules, with 10 µg mL^{-1} hydrochlorothiazide added to each mixture. These solutions were injected for analysis of the amounts of the drugs present.

A typical chromatogram obtained from a sample solution is shown in *Fig. 3*.

Results and Discussion

Method Development

The objective of the study was to develop simple and reproducible isocratic HPLC method using readily available chemicals and reagents. Modification of the aqueous and the organic components of the mobile phase resulted in satisfactory separation of drug components and IS with a 45:30:30 (v/v) mixture of 30 mM aqueous ammonium sulphate (pH 5.5), methanol, and acetonitrile at a flow rate 1 mL min^{-1}. Alteration of mobile phase pH (3 to 6) and use of 50 mM sodium orthophosphate, ammonium acetate, or heptanesulphonic acid, as ion-pairing reagent, with organic phases acetonitrile and methanol in different proportions resulted in no significant improvement in resolution or peak shapes of the drugs and IS.

The optimum wavelength for detection was 230 nm, as shown in *Fig. 3*. Acquisition of 3D spectra (*Fig. 4*) showed no indigenous interfering components eluted at the retention times of the drugs and IS.

Validation of the Method

The method was validated, in accordance with ICH guidelines, for linearity, accuracy, precision, specificity, sensitivity, LOD, LOQ, ruggedness, and robustness.

Linearity

Linearity was investigated by analysis of the calibration solutions described above. The standards were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solution. Linear correlations were obtained over the ranges studied, with correlation coefficients ≥0.99 for the drugs. For tablets the regression equations were $y = 0.0252x + 0.0046$ ($R^2 = 0.998$) for AMB and $y = 0.0461x + 0.0036$ ($R^2 = 0.999$) for CTZ. For capsules the regression equations were $y = 0.0268x + 0.0009$ ($R^2 = 0.999$) for AMB and $y = 0.0424x + 0.0035$ ($R^2 = 0.999$) for LCTZ.

Precision

The precision of the method was assessed by replicate ($n = 6$) analysis of both pharmaceutical preparations. Precision was also studied by analysis of standard solutions containing both drugs at concentrations covering the entire calibration range. Intra-day precision was determined by analysis of the solutions three times on the same day. Inter-day precision was assessed by analysis of the solutions on three different days over a period of one week.

Accuracy

Accuracy was determined by the method of standard additions at three different levels, by multiple level recovery studies. Solutions containing 12 µg mL^{-1} AMB, 1 µg mL^{-1} CTZ for tablets and 15 µg mL^{-1} AMB and 1 µg mL^{-1} LCTZ for capsules was prepared from the stock solutions and spiked with amounts of the standard drugs equivalent to 80, 100, and 120% of the amounts present in the original solution. These solutions were then analyzed for recovery studies. Results from determination of precision and accuracy are presented in *Table I*.

Specificity and Sensitivity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interferences were detected at the retention times of AMB, CTZ, LCTZ, or hydrochlorothiazide in sample solutions. Peak purity for AMB, CTZ, LCTZ and hydrochlorothiazide was

tested by comparing spectra acquired at the start (S), apex (A), and end (E) of the peaks.

Sensitivity for AMB, CTZ, and LCTZ was estimated as limit of detection (LOD) and limit of quantification (LOQ). These were calculated by use of the equations LOD = 3.3 × N/B and LOQ = 10 × N/B, where N is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of the noise, and B is the slope of the corresponding calibration plot. LOD and LOQ values are reported in *Table I*.

Table I. Results from validation and system-suitability studies

Method characteristic	Ambroxol HCl	Cetirizine HCl	*levo*-Cetirizine diHCl
Linear range (μg mL^{-1})	3–20	1–11	1–11
Correlation coefficient	0.998	0.999	0.999
Standard deviation	0.155	0.172	0.162
Theoretical plates	5386	10085	11265
Resolution	0.210	1.344	1.413
Asymmetry	0.99	1.00	1.00
Accuracy (%)	98.5	99.6	101.2
LOD (ng mL^{-1})	20	12	12
LOQ (ng mL^{-1})	60	38	38
Tailing factor	1.01	0.99	1.0
Precision RSD (%)			
Repeatability day 1	0.56	0.62	0.42
Intermediate precision day 2	0.64	0.72	0.55

Recovery

Recovery was determined by spiking the formulations with standards of each drug equivalent to 80, 100, and 120% of the amounts originally present. Average recoveries ranged from 98 to 102%, as reported in *Table II*.

Table II. Results from analysis of formulations and from recovery studies

Formulation[a]	Labelled amount (mg per dose)	Amount found (mg per dose)	Recovery (%, mean ± SD, n = 6)
Relent tablets			
Ambroxol HCl	60	60.50	101.80 ± 0.486
Cetirizine HCl	5	4.99	99.98 ± 0.240
Laveta-A capsules			
Ambroxol HCl	75	75.20	98.00 ± 0.526
levo-Cetirizine HCl	5	5.01	100.01 ± 0.270

[a]Relent tablets – Dr Reddy's Laboratories, Baddi; Laveta-A capsules – Alembic, Alembic Road, Vadodara

Stability

To demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results showed that for both solutions, the retention times and peak areas of AMB, CTZ and LCTZ remained almost unchanged (RSD <2.0%) indicating that no significant degradation occurred within this period, i.e. that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process. Sample solutions were then stored at 4 and 25°C and checked after three days of storage. When results were compared with those from freshly prepared samples in each case no significant degradation occurred within the indicated period.

Ruggedness and Robustness

The ruggedness of the method was determined by using different instruments (Shimadzu LC-10AT and Water's Breeze) and different columns of similar type (Hypersil C18 and Phenomenex Luna C18). The robustness of the method was determined by making slight changes in the chromatographic conditions (buffer pH ±0.5, flow rate ±0.2 min). Again there was no marked change in the chromatograms. These results indicated the method was rugged and robust with regard to these conditions. When mobile phase composition was changed by ±5%, however, proper resolution could not be achieved; separation of the drugs and the internal standard was very sensitive to mobile phase ratio.

System suitability data (number of theoretical plates per meter, resolution, peak asymmetry, and tailing factor for AMB, CTZ and LCTZ are reported in *Table I*. The standard deviations of the data were within ±3% range during routine performance of the method.

Conclusion

Only individual analytical methods for ambroxol HCl, cetirizine HCl, and *levo*-cetirizine HCl are reported in the literature. We have developed a method for simultaneous analysis of AMB with CTZ and AMB with LCTZ in combined solid dosage forms.

The proposed RP-HPLC method for simultaneous estimation of ambroxol hydrochloride, cetirizine hydrochloride and *levo*-cetirizine dihydrochloride in combined solid dosage forms is accurate, precise, and reproducible. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations, and for dissolution studies. It can be used for bioequivalence studies, in plasma, for both formulations.

Acknowledgment

The authors thank Apex Pharmaceuticals (A.P.) for providing gift samples of *levo*-cetirizine, hydrochlorothiazide, and ambroxol hydrochloride, Cetirizine hydrochloride was procured from (Franco-Indian Pharma, Mumbai, India). We also thank Mr Supe, Drug Inspector, Mr S. Rajan, and Mr S. Muralidharan of Cadrat for precious help. The authors are grateful to "His Holiness Jagadguru Sri Sri Shivarathree Deshikendra Mahaswamigalavaru" of Sri Suttur Mutt, Mysore, for providing facilities to conduct this work.

References

[1] The Merck Index – An Encyclopedia of Chemicals, Drugs, and Biologicals, 11th edn, Merck Research Laboratories, Whitehouse Station, New Jersey, 1989, pp. 62, 310
[2] J.H. Day, A.K. Eillis, and F. Rafeiro, Drugs Today (Barc), **40**, 415 (2004)
[3] Z. Dincer, H. Basan, and N.G. Goger, J. Pharm. Biomed. Anal., **31**, 867 (2003)
[4] J.E. Koundourellis, E.T. Malliou, and T.A. Broussali, J. Pharm. Biomed. Anal., **23**, 469 (2000)
[5] J. Drozd, H. Hopkala, G. Misztal, and B. Paw, Acta. Pol. Pharm., **59**, 3 (2002)
[6] A.F. El Walily, M.A. Korany, A. El Gindy, and M.F. Bedair, J. Pharm. Biomed. Anal., **17**, 435 (1998)

[7] M.S. Arayne, N. Sultana, and F.A. Siddiqui, Pak. J. Pharm. Sci., **18**, 7 (2005)
[8] A.M. Jaber, H.A. Al Sherife, M.M. Al Omari, and A.A. Badwan, J. Pharm. Biomed. Anal., **36**, 341 (2004)
[9] B. Paw, G. Misztal, H. Hopkala, and J. Drozd, Pharmazie., **57**, 313 (2002)
[10] A. Jelinska, B. Stanisz, M. Zajac, W. Musial, and A. Ostrowicz, Acta. Pol. Pharm., **57**, 171 (2000)
[11] J. Moncrieff, J. Chromatogr. **583**, 128 (1992)
[12] J. Macek, P. Ptacek, and J. Klima, J. Chromatogr. B, **736**, 231 (1999)
[13] M.F. Zaater, Y.R. Tahboub, and N.M. Najib, J. Pharm. Biomed. Anal., **22**, 739 (2000)
[14] M.R. Morita, D. Berton, R. Boldin, F.A.P. Barros, E.C. Meurer, A.R. Amarante, D.R. Campos, S.A. Calafatti, R. Pereira, E. Abib Jr, and J. Pedrazolli Jr, J. Chromatogr. B, **862**, 132 (2008)
[15] ICH, Q2A: Text on Validation of Analytical Procedures, International Conference on Harmonization. October 1994
[16] ICH, Q3B: Validation of Analytical Procedures: Methodology, International Conference on Harmonization, November 1996
[17] L.R. Snyder, J.J. Kirkland, J.L. Glejch (Eds) Practical HPLC Method Development, Wiley–Interscience, New York, 1988, pp. 402–438